EVIDENCE FOR HORMONE-CONTAINING GRANULES IN SINUS GLANDS OF THE FIDDLER CRAB UCA PUGILATOR

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It is known that in neurosecretory systems the products of secretion are stored in axon terminations where they aggregate in particles or granules (Scharrer and Scharrer, 1954; Welsh, 1955).

Some recent electron microscope studies have shown a constancy in the appearance of such structures in the neurohypophysis of different vertebrates (Duncan, 1955, 1956). The size of these granules varies from 0.1 to 0.3 micron, and in the neurohypophysis of the rat they seem to be bounded by a delicate membrane (Palay, 1955).

Through the differential centrifugation technique for isolation of mitochondria and other particles of the cells, Hillarp, Lagersted and Nilson (1953) and Blaschko and Welch (1953) could obtain a fraction of granules which is responsible for 80 to 90% of the total adrenaline and noradrenaline present in the adrenal medulla of cattle. Further, Hillarp and Nilson (1954) and Blaschko, Hagen and Welch (1955), doing physiological experiments with the separated granular fraction, obtained information concerning the nature of the granules containing the catechol amines. Similar results were obtained for the granules containing vasopressin and oxytocin in the posterior pituitary of the rat (Pardoe and Weatherall, 1955). The observations of the several authors, above cited, strongly support the assumption that the granules have a semipermeable membrane of a lipid or lipo-protein nature. The granules, which are stable in isotonic solutions of saline or sucrose, release their hormone when treated by agents which are known to damage biological membranes.

In the invertebrates, especially among insects and crustaceans, some neurosecretory systems are very well known, and the study of the granules in these systems might give valuable information concerning such storage particles. A good structure for these studies is the "sinus gland" of the crustaceans. A sinus gland in each eyestalk is the storage-release organ for several neurohormones of the crustaceans. They are formed by the axon terminations of neurosecretory cells localized in the X-organ, in the brain and in other parts of the central nerve system (Passano, 1951a, 1951b; Bliss and Welsh, 1952; Bliss, Durand and Welsh, 1954). The axon terminations in sinus glands are filled with granules 0.1 to 0.3 micron in diameter (Potter, 1956) which can be seen in living preparations (Passano, 1952).

The aim of the present work was to show that the granules in sinus glands are really the depots of neurosecretory materials and that they behave like similar structures found in the neurosecretory systems of vertebrates.

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Material and Methods

For this purpose the study of one of the chromatophorotropic hormones stored in sinus glands was chosen. According to the usual technique for isolation and preservation of mitochondria (Hogeboom, Schneider and Palade, 1948) the sinus glands were homogenized in isotonic solutions of sucrose. The homogenates before and after several treatments were injected into test animals for an estimation of the activity of the hormone in the different cases.

Preparation of the homogenates

Sinus glands of the fiddler crab, *Uca pugilator,* from Florida, were used in these experiments. With the aid of a dissecting microscope the sinus glands were isolated from the adjacent tissues immediately after cutting the eyestalk of the crabs, and were placed in solutions of cold 1.3 *M* sucrose, which, according to Abramowitz and Abramowitz (1938), is isosmotic with the blood of *Uca.* In each experiment four or more sinus glands were homogenized in one ml. of isotonic sucrose, in the Elvehjem homogenizer for three minutes. After homogenization more sucrose was added according the requirement of the experiment. A part of the homogenate was then kept at 2° C. until the moment of the experiment, and the remainder was submitted to different treatments. Before being injected into the test animals all homogenates were diluted in isotonic sucrose, or sea water to make the same final concentration. All the procedures were carried out in the cold at 2° C. For details, see below.

Assays

The activity of the black chromatophore-dispersing hormone in the different homogenates was tested in isolated legs of *Uca pugnax.* It was observed that in legs of *Uca pugilator* when they are separated from the body, the black chromatophores disperse gradually. Such dispersion may be explained by a direct effect of light on the chromatophores, since legs isolated and kept in sea water in the dark do not show this phenomenon. A direct effect of light on the chromatophores of *Uca pugilator* has been already observed by Brown and collaborators (Brown and Sandeen, 1946, 1948; Brown, Guyselman and Sandeen, 1949). For this reason in the present experiments the legs of *Uca pugnax* which do not show this behavior were used.

Uca pugnax were destalked 24 hours before the experiment so that at the time of the experiment the black chromatophores were in the stage of maximal concentration. The legs were cut off at the level of the ischial segment, and were placed in 5 ml. of sea water in Syracuse dishes. Each leg received an injection of 0.01 ml. of homogenate, and the dispersion of the black chromatophores was observed every ten minutes for one hour. *Uca pugilator* was used for experiments in which the homogenates were tested in the whole animal. In these cases, each animal received 0.1 ml. of homogenate and the stages of the chromatophores were observed for several hours.

Preliminary attempts were made to remove granules from the homogenates by centrifugation.

All the results are presented in graphs according to Hogben and Slome (1931),

where 1 represents maximal concentration of the chromatophores, 5 maximal dispersion, and 2, 3 and 4, intermediate stages.

Results

I. Hormone in granules and in cytoplasm

The homogenate of 4 sinus glands in 1 ml. of 1.3 *M* sucrose was divided; one-half of the suspension received 4.5 ml. of distilled water and was kept at 2° C.; the other half was kept undiluted at the same temperature. After one-half hour, the homogenate in sucrose was diluted with sucrose, and that with added distilled

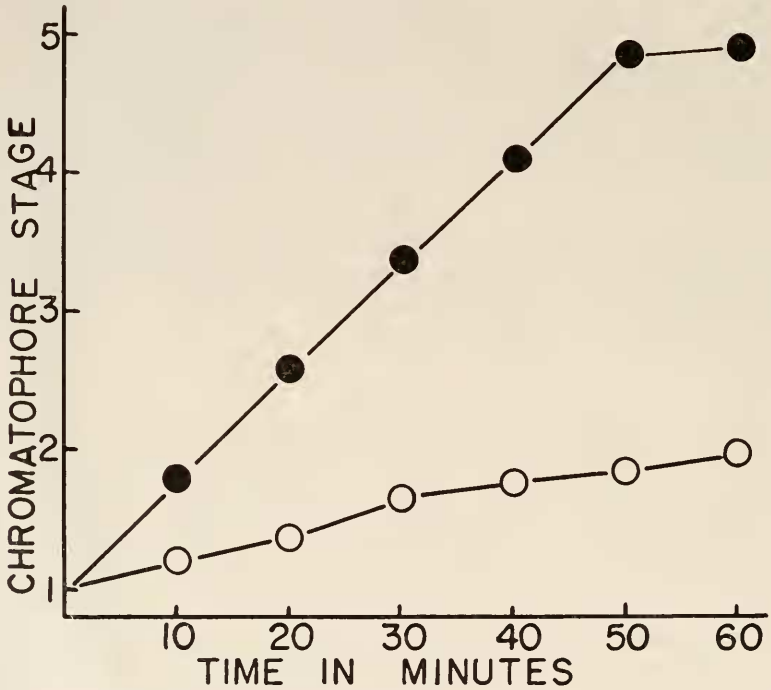

Figure 1. Response of the black chromatophores of isolated legs of *Uca pugnax* to injections of homogenates of sinus glands of *Uca pugilator*: ●, homogenates in distilled water; ○, homogenates in 1.3 *M* sucrose. Each point in the graph represents the average of 20 experiments.

water was diluted with sea water to make a final concentration of 0.02 sinus gland per ml. Every time that distilled water was added to the homogenates, the final dilution was made in sea water; these homogenates throughout the paper will be called "homogenates in distilled water," to shorten the explanation.

As one can see in Figure 1, the homogenate in distilled water caused maximal dispersion of the chromatophores in the legs of *Uca,* and that in sucrose exhibited only a small effect. These results show that in isotonic sucrose the black chromatophore-dispersing hormone is present in large part in a state in which it cannot act; whereas in distilled water it seems to be free in solution and able to induce the dis-

persion of the chromatophores. This evidence supports the view that the hormone is contained in granules, which in isotonic sucrose remain intact and in distilled water release the hormone into the solution.

If that were the case it would be possible to separate a fraction of granules containing hormone, using the usual technique of differential centrifugation. To avoid the high density of a medium like 1.3 *M* sucrose, 16 sinus glands were homogenized in one ml. of a mixture of 25% of 1.3 *M* sucrose and 75% of sea water, which has been demonstrated to be as effective in preserving the granules as pure sucrose. The homogenate, after dilution to make 10 ml., was centrifuged at 800 × gravity

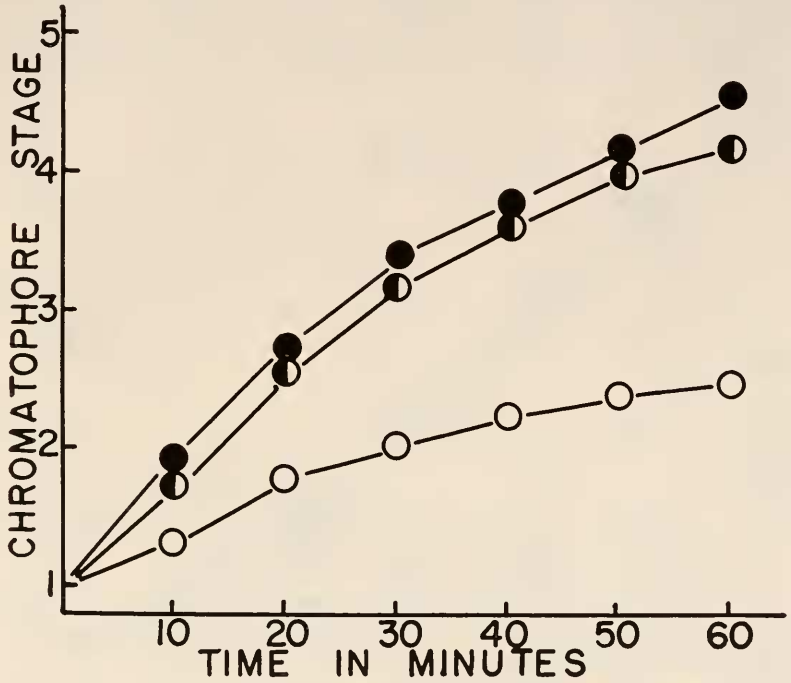

FIGURE 2. Response of black chromatophores of *Uca* to injections of homogenates of sinus glands in isotonic sucrose after centrifugation: ●, "supernatant A" (low speed); ◐, "supernatant B" (high speed); ○, "sediment C" (high speed).

for 30 minutes for separation of unbroken cells, nuclei, etc. No visible sediment was observed after this slow-speed centrifugation, so the whole solution was decanted. Part was set aside, as "solution A," and the rest was centrifuged at 20,000 × gravity for 30 minutes. No sediment was observed this time either. The whole solution was decanted carefully and taken as "solution B." Then one ml. of distilled water was added to the centrifuged tube and was stirred and the tube walls were scraped with a spatula. After 15 minutes 9 ml. of sea water were added to make 10 ml., and this solution was called "solution C." Part of the solution A and B was treated with distilled water and all three solutions were finally diluted to the concentration of 0.02 sinus gland per ml. Both solutions A and B showed the same

effect on the chromatophores of legs of *Uca pugnax*. No significant loss of activity was observed in solution B after the high speed centrifugation. However, solution C, the suspension of a presumably invisible sediment, caused a small effect on the chromatophores (Fig. 2). This fact is indicative of some sedimentation of granules and from these results it is not possible to infer how much of the hormone is present in granules and how much is found free in the homogenate. It is probable that for a complete sedimentation a longer and higher-speed centrifugation is necessary.

An indication of the percentage of hormone contained in granules in isotonic sucrose is given by the analysis of the activity of homogenates in isotonic sucrose and distilled water after a series of dilutions. Figure 3 shows the results of injections of 0.01 ml. of homogenates of 2 sinus glands in 1 ml. of 1.3 *M* sucrose and in 1 ml. of distilled water diluted 10, 100 and 1000 times in 1.3 *M* sucrose and sea

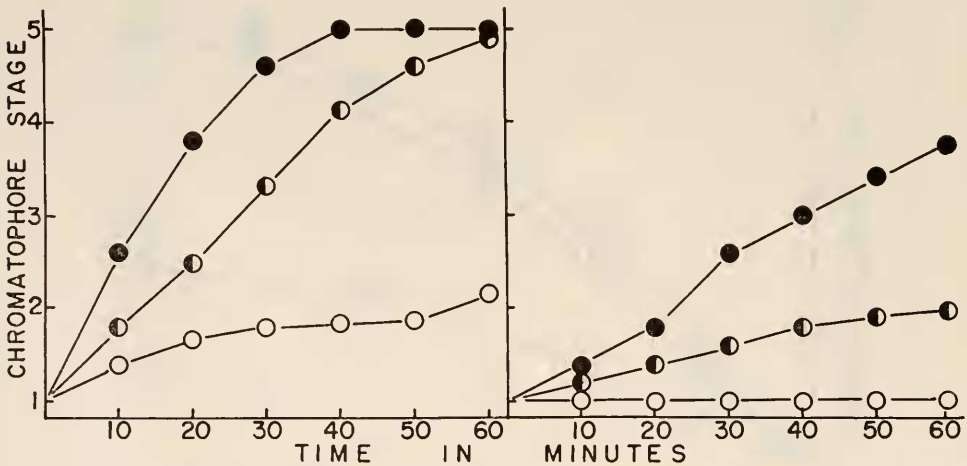

FIGURE 3. Response of black chromatophores of *Uca* to injections of homogenates of sinus glands in distilled water (left) and in 1.3 *M* sucrose (right) in different concentrations: ●, 0.2; ◐, 0.02; and ○, 0.002 sinus glands per ml.

water, respectively. It appears that in isotonic solution only less than 10% of the hormone is found free in the suspension, since the effects of the homogenates in sucrose are smaller than those of the homogenates in distilled water, ten times more diluted.

II. Effect of several treatments on the release of the hormone

1. Effect of the tonicity of the medium. From the homogenates of 8 sinus glands in 2 ml. of 1.3 *M* sucrose, 7 samples of 0.25 ml. each were separated. The addition of 9.75 ml. of 0.9, 0.8, 0.7, 0.65, 0.32 *M* sucrose was made to a series of 5 tubes and to a sixth, 4.75 ml. of distilled water were added. After one hour at 2° C., the solutions were diluted in 1.3 *M* sucrose to the final concentration of 0.02 sinus gland per ml. and were tested on isolated legs. Figure 4 illustrates the activity of the hormone in the different solutions. As the tonicity of the medium de-

creases there is a liberation of hormone which, to some extent, is proportional to the concentration of sucrose, from 1.3 to 0.7 *M*. In 0.65 and 0.32 *M* solutions there seems to be a complete release since the activity of the hormone in these two latter concentrations is as great as that of homogenate in distilled water. The action of distilled water after 15 minutes standing is as effective as after 30 minutes. This fact shows that the release in distilled water is rapid. The granules in this respect, like red blood cells and mitochondria under the same conditions, appear to act as osmometers.

2. Effect of different solutions. One group of experiments was performed to determine whether the granules containing the hormone are also stable in isotonic

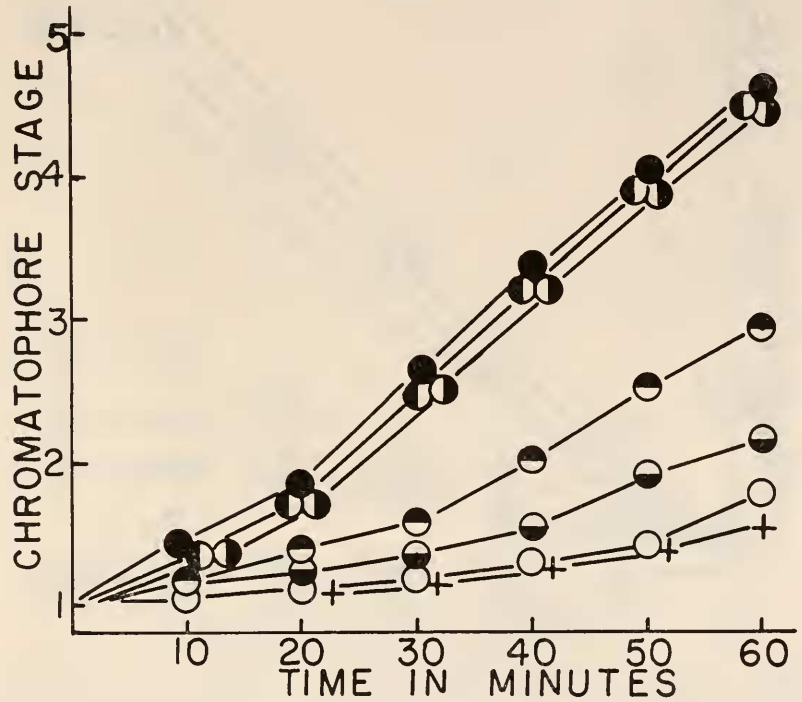

FIGURE 4. Response of black chromatophores of *Uca* to injections of homogenates of sinus glands in different concentrations of sucrose solutions: +, 1.3; ○, 0.9; ◒, 0.8; ◓, 0.7; ◑, 0.65; and ◐, 0.32 *M*; ●, homogenate in distilled water.

solutions of electrolytes. Samples of 0.5 ml. of homogenates in 1.3 *M* sucrose were held for 30 minutes at 2° C. with the addition of 4.5 ml. of the following: distilled water, sea water, sodium chloride and potassium chloride. The sodium chloride was either isotonic with sea water (0.54 *M*) or isotonic with 1.3 *M* sucrose (0.78 *M*). Figure 5 shows that sea water and isotonic salt solutions produce a large and rapid release of hormone. This fact indicates that the simple dilution in isotonic electrolyte solutions is sufficient to provoke alterations in the granules very similar to those observed by lowering the tonicity of the medium. However, in electrolyte

solutions to which an equal part or a fourth part of isotonic sucrose is added, the granules remain largely intact. The activity of the hormone in these media (Fig. 5) is comparable to that in isotonic sucrose.

In another group of experiments an effort was made to find the best medium for preservation of the granules. Watanabe and Williams (1953) have shown that 2.5% bovine plasma albumin in isotonic potassium phosphate buffer at pH 7 is a good medium to preserve mitochondria of insect muscles. In the following experiments, besides the 1.3 *M* sucrose, a mixture of 25% 1.3 *M* sucrose and 75% sea water was also used, as well as 2.5% bovine plasma albumin in 0.54 *M* potassium

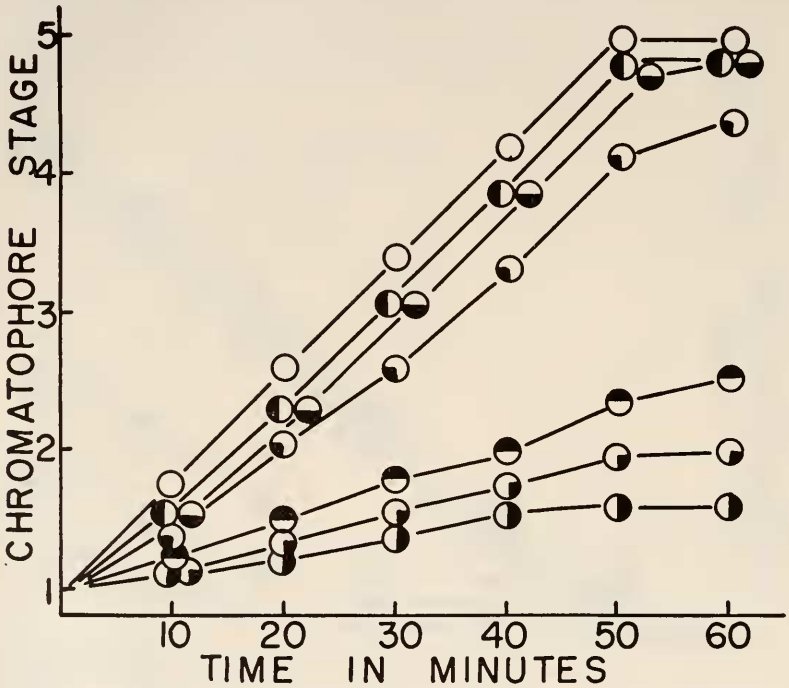

FIGURE 5. Response of black chromatophores of *Uca* to injections of homogenates of sinus glands in different solutions: ○, distilled water; ◐, sea water; ◒, 0.78 and 0.54 *M* NaCl; ◔, 0.54 *M* KCl; ◓, ◔, and ◑, NaCl, KCl and sea water in a mixture with 25% of 1.3 *M* sucrose.

phosphate at pH 7. The homogenates of sinus glands in these three media were kept at 2° C. and at different times were diluted in 1.3 *M* sucrose and assayed using legs of *Uca* (Fig. 6). After 6 hours of incubation in these media, the activity of the black chromatophore-dispersing hormone is insignificant, and after 24 hours only a slight effect was observed. That the hormone was preserved in the granules was shown by the following procedure. After 24 hours the homogenates were heated for 5 minutes in boiling water and diluted in sea water. After this treatment all the solutions produced a maximal dispersion of the chromatophores, comparable to that caused by homogenates in distilled water. Thus, the three different media used seem to be equally efficient in keeping the granules intact.

3. Effect of heat, and of freezing and thawing. A release of hormone from the granules was observed when homogenates of sinus glands in isotonic sucrose were kept at room temperature for several hours. However, homogenates in 1.3 *M* sucrose when heated for 5 minutes at 70° C. or in boiling water showed only a slightly greater activity than the original homogenate without this treatment (Fig. 7).

Freezing at − 10° C. and thawing to room temperature three times in succession was more effective than heating, but even so, the release of the hormone was not the same as when homogenate was merely diluted in distilled water (Fig. 7).

4. Effect of detergents and digitonin. Detergents and digitonin did not give a complete release of hormone from the granules. Samples of 0.25 ml. of homoge-

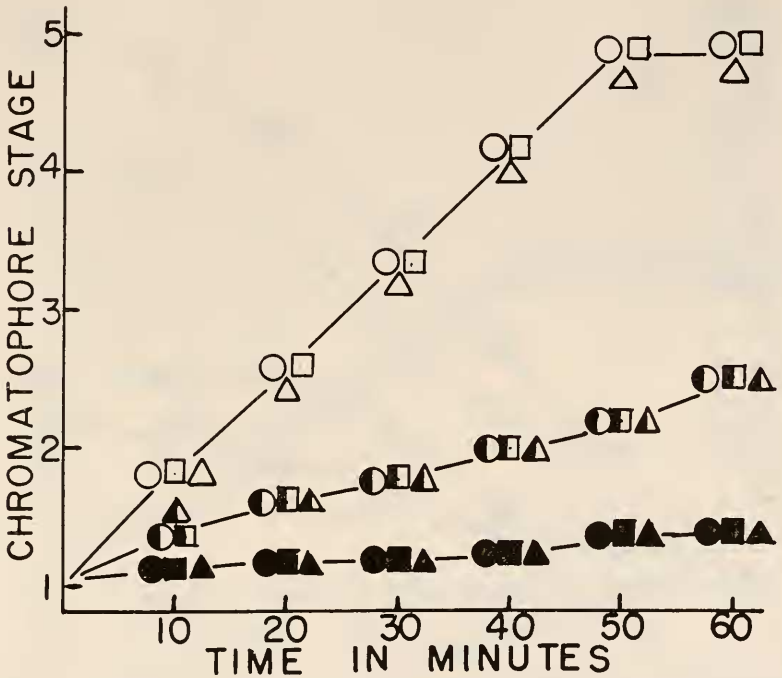

FIGURE 6. Response of black chromatophores of *Uca* to injections of homogenates of sinus glands in different media. Circles, homogenate in 5% bovine plasma albumin in 0.54 *M* potassium phosphate buffer; squares, homogenate in 1.3 *M* sucrose; triangles, homogenate in 25% 1.3 *M* sucrose plus 75% sea water. ●, ■, ▲, homogenates kept 6 hours and ◐, ◧, ◭, 24 hours at 2° C.; ○, □, △, after being kept 24 hours at 2° C., the homogenates were heated and diluted in sea water.

nates in 1.3 *M* sucrose were maintained for one hour at 2° C. with 1.75 ml. of 10^{-3} *M* concentration of the following substances: sodium lauryl sulfonate (Duponol); sodium desoxycholate, saponin and digitonin, in 1.3 *M* sucrose. After the required dilution of the homogenates for the bio-assays, the concentration of the detergents and digitonin was 10^{-5} *M*. When control legs of *Uca* or the whole control animal received injections of the detergents and digitonin in such concentration, no effect on the chromatophores was observed. Therefore, the dispersion following the injections of homogenates in sucrose plus detergents is attributed to the hormone present in the solutions.

The detergents employed and digitonin provoked only a partial release of hormone (Fig. 8). Part of the homogenate plus desoxycholate after one hour of incubation was heated and diluted in sea water, and greater activity was seen after this treatment.

III. Inactivation of the hormone

In some experiments the homogenates of sinus glands in distilled water were injected into the whole crab (*Uca pugilator*). Figure 9 shows the degree and the

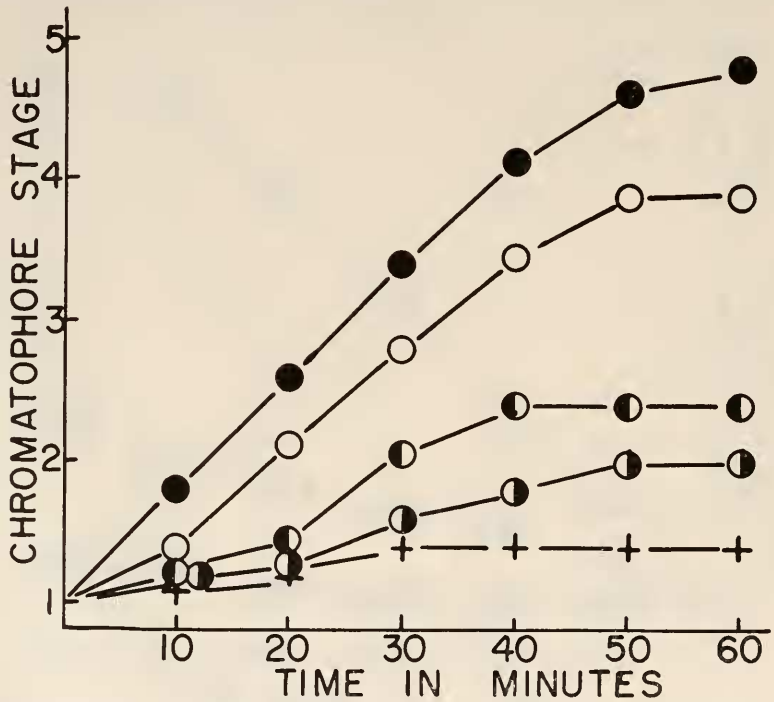

FIGURE 7. Response of black chromatophores of *Uca* to injections of homogenates of sinus glands in isotonic sucrose before, +, and after heating at 70° C., ◑, and in boiling water, ◐; and after freezing and thawing, ○. Homogenate in distilled water, ●.

duration of the dispersion of chromatophores in relation to the concentration of the homogenates. The injection of 0.1 ml. of a homogenate of 0.2 sinus gland per ml., *i.e.*, the injection of an amount corresponding to 0.02 sinus gland, is enough to cause a maximal dispersion of the chromatophores in almost 30 minutes and only four hours later have the chromatophores reached the stage of complete concentration again. It is interesting to notice that the time required for normal dark *Uca* to become pale after eyestalk removal is three to four hours. At all concentrations of homogenates dispersion was found to require less time than concentration of pigment within the chromatophores. The elimination of the hormone seems to be a very slow process. Even an injection corresponding to 0.001 sinus gland (open

circles in Fig. 9) induces an effect which disappears completely only after three hours.

In order to obtain some information about the inactivation of the hormone, homogenates of sinus gland in distilled water were incubated with extracts of hepatopancreas, hypodermis and muscle and with one ml. of blood of *Uca*. The extracts were prepared by homogenizing one hepatopancreas, the muscle of one claw, and the hypodermis of the branchiostegites separately, in one ml. of sea water. The blood was removed at the junction of the body and the fourth walking

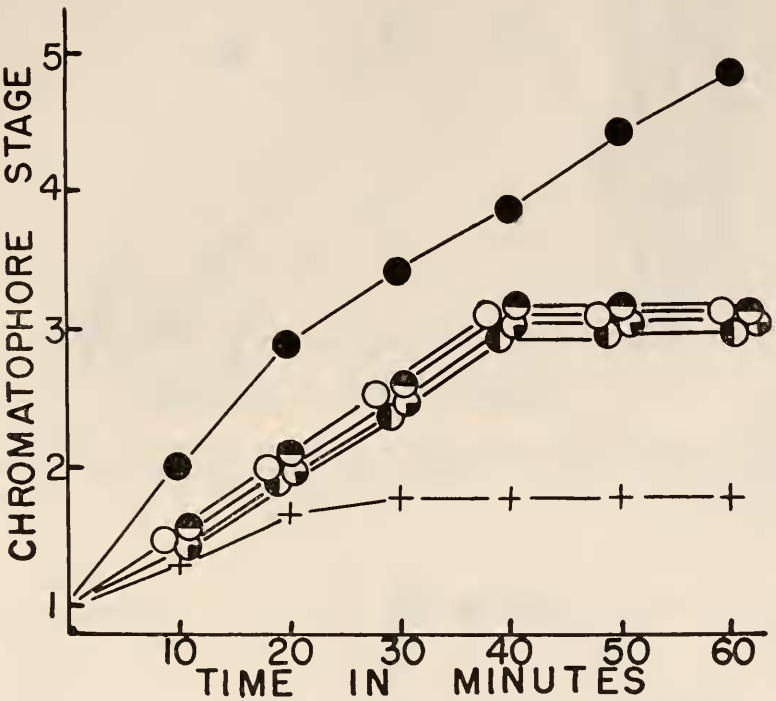

FIGURE 8. Response of black chromatophores of *Uca* to injections of homogenates of sinus glands in isotonic sucrose before, +, and after treatment with detergents and digitonin. ◐, Duponol; ◓, saponin; ○, sodium desoxycholate; ◔, digitonin; ●, sodium desoxycholate plus heat and dilution in sea water.

leg, with the aid of a glass pipette. The only extract which caused a complete inactivation of the black chromatophore-dispersing hormone was that of hepatopancreas. After one hour of incubation with extracts of hypodermis or muscle, or with blood, at room temperature, no decrease in the activity of the hormone was observed (Fig. 10a).

The enzyme in the hepatopancreas responsible for its action might be a proteolytic one, since the same effect was obtained when homogenates of sinus glands in distilled water were incubated at 37° C. for one hour with some crystals of chymotrypsin (Fig. 10b). These results suggest that the black chromatophore-dispersing

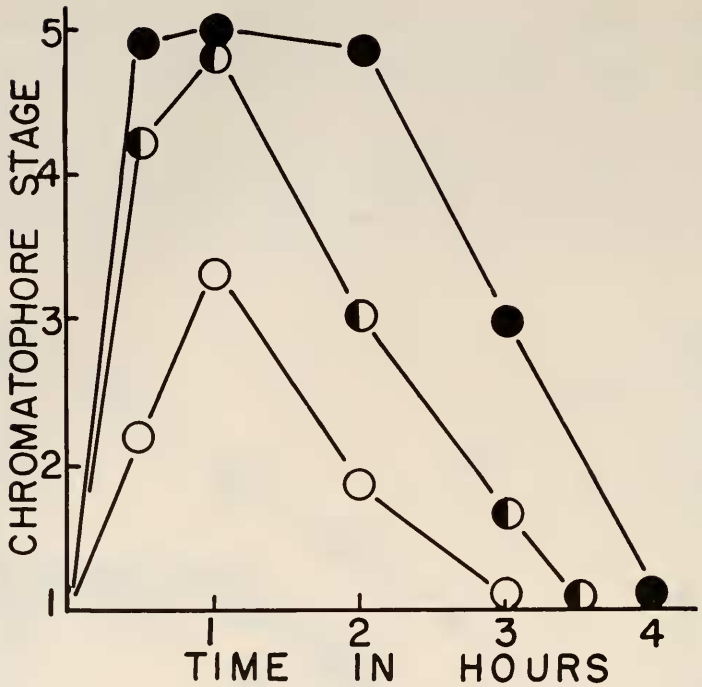

FIGURE 9. Response of black chromatophores of the whole *Uca pugilator* to injections of 0.1 ml. of homogenates of sinus glands in distilled water, in different concentrations: ●, 0.2; ◐, 0.02; ○, 0.001 sinus gland per ml.

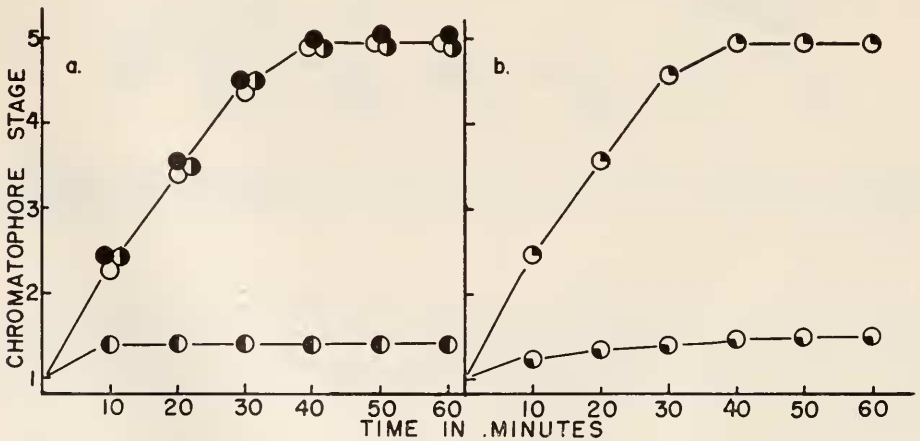

FIGURE 10. Response of black chromatophores of *Uca* to injections of homogenates of sinus glands in distilled water before and after incubation with extracts of different tissues for one hour at room temperature and after incubation with chymotrypsin for one hour at 37° C.: ●, muscle; ○, blood; ◑, hypodermis; ◐, hepatopancreas; ◔, distilled water; and ◌, chymotrypsin.

hormone is a polypeptide, but the acceptance of this hypothesis depends upon further experiments.

Discussion

The experiments in section I indicate that in the crab, *Uca pugilator,* the black chromatophore-dispersing hormone is stored in sinus glands within the granules. This assertion is supported by the following observations. First, homogenates of sinus glands in isotonic sucrose have only a small effect on the chromatophores of legs of *Uca pugnax.* These homogenates diluted in distilled water cause a maximal dispersion of the chromatophores, indicating a more or less complete release of the hormone. Second, a sedimentable fraction containing hormone was obtained by centrifugations at the speed of 20,000 × gravity. This centrifugation caused only a partial sedimentation of granules. However the analysis of the activity of homogenates in isotonic sucrose and in distilled water after a series of dilutions shows that the homogenates in sucrose are as effective as those in distilled water ten times more diluted, indicating that only 10% or less of the total amount of hormone is present free in the solution, the other 90% remaining in the granules. Whether this free hormone is already present in sinus glands *in vivo,* or whether it is the effect of the disruption of some granules during the process of homogenizing, is not known. Hillarp, Lagersted and Nilson (1953) have observed that at increased duration of homogenization the catechol content of the granules of the adrenal medulla cells decreases. Berthet and De Duve (1951) have also found that a partial damage to the mitochondria containing acid phosphatase is caused by the process of homogenizing liver tissue. This may be the case with the homogenates of sinus glands.

The effect observed by lowering the tonicity of the medium reinforces the evidence of the presence of the chromatophore-dispersing hormone in granules, and suggests the existence of a semipermeable membrane for the granules. The rapid release of hormone observed when the tonicity of the medium decreases suggests that there is a lysis of the granules, by rapid entrance of water.

The membrane of the granules seems to be freely permeable to ions like sodium and potassium, because the solutions of isotonic sodium chloride, potassium chloride, or sea water cause an immediate and marked release of hormone from the granules. Hillarp and Nilson (1954) have found that the granules of the adrenal medulla can be suspended in sucrose or in certain isotonic electrolyte solutions without a considerable release of catechol amines. Blaschko, Hagen and Welch (1955), however, have observed that in NaCl or KCl an appreciable liberation of adrenaline occurs. Pardoe and Weatherall (1955) also have obtained liberation of vasopressin and oxytocin from granules of the posterior pituitary of rats, by simple dilution in saline of the suspensions of granules in isotonic sucrose. Isotonic saline solutions have been demonstrated to afford only transient osmotic protection for mitochondria of the rat liver (Berthet, Berthet, Appelman and De Duve, 1951; Appelman and De Duve, 1955) and for mitochondria of insect muscle (Watanabe and Williams, 1953). The authors above cited observed also that in media where part of the saline is replaced by isotonic sucrose, the mitochondria are very stable. Similarly, the granules of sinus glands are equally stable in pure sucrose, in a mixture of 25% isotonic sucrose and 75% isotonic salines, and in 2.5% bovine plasma albumin in 0.54 *M* potassium phosphate buffer at pH 7.

Heating, freezing and thawing, and the action of detergents have been proved efficient treatments to release physiologically active substances from granules (Hillarp and Nilson, 1954; Pardoe and Weatherall, 1955). In the case of granules of sinus glands, all these treatments induce a more or less appreciable release of hormone but none of them is sufficient to cause a complete liberation of hormone from the granules.

The hormone in the homogenates in isotonic sucrose after heating, freezing and thawing and after the action of detergents is still present either inside the granules or in such combination that it can not be active. This was proved by the experiments in which parts of the homogenates after these treatments were diluted in sea water, and greater activity was then observed.

These observations may suggest the following hypothesis: that inside the granules the chromatophore-dispersing hormone is found in two forms, bound to a large molecule and as free small molecules. By heating, freezing and thawing and by the action of detergents, the membrane of the granules suffers some disruption, permitting only the passage of the small molecules to the solution. In hypotonic and saline media, which cause a lysis of the granules, all the molecules are present free in the solution. One has to admit also that the hormone is active in both forms, or that once free in the solution, the large molecules disintegrate into the smaller ones. This could explain the different activity of the homogenates of sinus glands in isotonic sucrose after these different treatments.

It is interesting to discuss here the results of Knowles, Carlisle, and Dupont-Raabe (1955) with the chromactivating substances of sinus glands and post-commissure organs of *Leander serratus,* and corpora cardiaca of *Carassius.* By electrophoresis of extracts of these organs they detected the presence of a substance, the "A-substance," which is relatively immobile at pH 7.5 and does not pass through cellophane membranes. This substance concentrates all the red chromatophores of *Leander.* When the extracts are left standing several hours at room temperature, the A-substance disintegrates into others, the a-substances, which have high mobility at pH 7.5 and pass freely through a dialysis membrane. The a-substances affect only the small red chromatophores of *Leander.* They observed also that only the a-substances are released by electrical stimulus of the commissure when the post-commissure organ is in a saline bath.

So, it is reasonable to believe that the dispersing hormone of *Uca* can also be found as large and small molecules and both be active on black chromatophores. But, of course, this is an assumption which depends upon further experiments in this subject.

Heating in boiling water does not cause loss in the activity of the chromatophore-dispersing hormone of *Uca.* Inactivation of the hormone can be achieved, however, by incubation of the homogenates of sinus glands with extracts of hepatopancreas and by the action of the enzyme chymotrypsin. These results suggest that the hormone is a polypeptide.

Carstam (1951) has found that extracts of hepatopancreas of crustaceans and molluscs, and extracts of liver of the guinea pig inactivate the pigment-concentrating hormone of *Leander adspersus,* but he could not obtain the inactivation of the hormone with trypsin. However, Knowles, Carlisle and Dupont-Raabe (1956) have obtained a complete inactivation of the "A-substance" from sinus glands and post-commissure organ of *Leander,* by a crystalline preparation of trypsin and also by a

prolonged acid hydrolysis. Östlund and Fange (1956) have suggested that a chromactivating substance from the eyestalk of *Pandalus* could be an aromatic amine, but in personal communication to Knowles and Carlisle (1956) they have stated that their more recent work indicates that this hormone may possibly be a polypeptide. So far, the studies concerning the nature of the chromactivating substances of crustaceans indicate that they are polypeptides. Porath, Roos, Landgrebe and Mitchell (1955) have isolated a melanophore-stimulating peptide from the pig-pituitary gland. Thus, also in vertebrates the chromatophorotropins seem to be peptides.

Carstam (1951) has also obtained the inactivation of the pigment-concentrating hormone by an enzyme present in the hypodermis of *Leander*. In *Uca pugilator,* in hypodermis as well as in the blood, there was not found an inactivating enzyme for the chromatophore-dispersing hormone.

The experiments where the homogenates of sinus glands in distilled water were injected into the whole *Uca pugilator* show that the response of the black chromatophores is a function of the concentration of the hormone. These results give also an idea about the amount of hormone liberated and its way of action in normal crabs. Insignificant amounts of the hormone (corresponding to 0.02 sinus gland) are enough to induce a maximal dispersion of the chromatophores for a long time. This shows that the elimination or destruction of such small quantities of hormone is a slow process. Stephens, Strickholm and Friedl (1956) have also observed that the dispersing hormone in *Uca* was present in the circulating blood of destalked assay animals in discernible amounts for approximately three hours after injection. Hence, it is reasonable to believe that the dispersing hormone is liberated into the blood in small quantities and eliminated by excretory processes without the interference of special enzymes for its inactivation.

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Summary

1. Homogenates of sinus glands in isotonic sucrose cause little dispersion of black chromatophores when injected into legs or whole *Uca*. A liberation of hormone occurs when homogenates of sinus glands in isotonic sucrose are diluted in distilled water. A fraction, sedimentable by high speed centrifugation, when resuspended in distilled water and injected into the test animals, induces a dispersion of the chromatophores. These results support the view that the black chromatophore-dispersing hormone is contained within granules in sinus glands.

2. The release of the hormone from the granules, obtainable by lowering the tonicity of the medium or by dilution in isotonic saline solutions, suggests that the granules possess a semipermeable membrane.

3. The release of the hormone from the granules is increased by heating, by freezing and thawing, and by the action of detergents and digitonin.

4. The black chromatophore-dispersing hormone may be a polypeptide, since it is inactivated by extracts of hepatopancreas and by chymotrypsin.

5. The rate of disappearance of the hormone from the blood of the crab is very slow.

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